Production and Processing of Spider Silk Proteins

Hardy, J. G., & Scheibel, T. R. (2009). Production and Processing of Spider Silk Proteins. *Journal of polymer science part a-Polymer chemistry*, *47*(16), 3957-3963. https://doi.org/10.1002/pola.23484

Published in:
Journal of polymer science part a-Polymer chemistry

Document Version:
Peer reviewed version

Title: Production and Processing of Spider Silk Proteins

Author List: John G. Hardy and Thomas R. Scheibel*

Institution Address: Lehrstuhl für Biomaterialien, Universität Bayreuth, Universitätsstr. 30, 95440 Bayreuth, Germany.

E-mail address of the corresponding author: Thomas R. Scheibel (thomas.scheibel@uni-bayreuth.de)

ABSTRACT:

Natural spider silk fibers have impressive mechanical properties (outperforming many man-made fibers) and are moreover biocompatible, biodegradable and produced under benign conditions (using water as a solvent at ambient temperature). The problems associated with harvesting natural spider silks inspired us to devise a method to produce spider silk-like proteins biotechnologically (the first subject tackled in this highlight); we subsequently discuss their processing into various materials morphologies, and some potential technical and biomedical applications.

Keywords: bioengineering, biomaterials, biomimetic, proteins, renewable resources

Authors biographies:

Dr. John Hardy was born in 1980. He studied chemistry at the University of Bristol, and carried out his doctoral studies under the supervision of D. K. Smith at the University of York in England. From 2006 to 2007 he was an Entente Cordiale postdoctoral fellow in the Lehn lab at the Université Louis Pasteur in France, and is currently an Alexander von Humboldt fellow at the Lehrstuhl für Biomaterialien at the Universität Bayreuth in Germany. His research interests include: biomimetics, biomaterials, novel polymers and supramolecular chemistry.

Professor Thomas Scheibel is chair of biomaterials at the Universität Bayreuth in Germany. He received both his Diploma of biochemistry and a Dr. rer. nat., from the Universität Regensburg in

Germany, and his habilitation from the Technische Universität München in Germany. He was a Kemper Foundation postdoctoral fellow and a DFG postdoctoral fellow at the University of Chicago. He received the junior scientist award of the center of competence for new materials in 2004. Together with a journalist he won the Promega award "Main Thing Biology" in 2005. He and his colleagues work on spider silk proteins won the second prize in the Science4life Venture Cup 2006. He also gained the Biomimetics award of the German Bundesministerium für Bildung und Forschung (BMBF) in 2006, and the "Innovation by nature award" of the BMBF in 2007. He is one of 10 recipients of the 2006 innovation tribute of the Bavarian prime minister, received the Heinz-Maier-Leibnitz Medal in 2007, and the Karl-Heinz Beckurts award in 2008. Thomas Scheibel is co-founder of the biotech company AMSilk GmbH.

INTRODUCTION

Spiders have evolved to be able to produce a variety of task-specific silks for catching prey (via trapdoors and webs), protection and preservation of their offspring and prey (in cocoon-like structures) and as lifelines to escape from predators.[1,2] More than 40,000 different species of spiders have been identified, of which approximately half catch their prey in webs. Orb webs[3-5] are a particularly interesting example of web design and they are constructed from five different types of task-specific silks, of which major ampullate and flagelliform silks are the most important (see figure 1).[6,7] In contrast to silkworms, it is impossible to farm most spiders in large scale due to their cannibalistic nature, therefore spider silks are typically obtained via harvesting of the silk at its point of application (in the case of spider webs great attention to detail is required to obtain uncontaminated samples); anaesthetization of single spiders followed by reeling of the silk fibers from its source gland (in the case of major and minor ampullate and cylindriform silks), or extraction of the spidroin directly from the gland in which it is produced (after killing the spider). In order to circumvent such complicated procedures, identification of the protein sequences has allowed the

recombinant production of genetically engineered analogues in sufficient yield and purity for application as high performance biopolymers.[8-16]

Major ampullate silk

Silk fibers made of proteins produced in the major ampullate silk gland (MA silk, molecular weight (MW) >300 kDa) have a very high-tensile strength (comparable to Kevlar) and moderate elasticity, and are used as a scaffold upon which to attach other silks during the construction of a web and as a lifeline when it is necessary to escape from a predator. MA silks have diameters between 1 and 20 μm (depending upon spider species) and have a core–shell type structure (depicted in figure 2).[17-20] The core contains two major proteins (Major Ampullate Spidroins 1 and 2) that are composed predominantly of glycine, alanine and proline (although the quantity of the latter varies significantly between species). Major ampullate spidroins of *Araneus diadematus* and *Nephila clavipes* spiders are reminiscent of block copolymers containing blocks of polyalanine and either $(GGX)_n$ (where X is typically tyrosine, leucine or glutamine) or GPGXX. The alanine-rich blocks are known to form β-sheet stacks that are responsible for the high-tensile strength of MA silks; whereas the blocks of $(GGX)_n$ form 3_{10}-helices , and the blocks of GPGXX form β-turn spirals imparting elasticity/flexibility to the proteins (see figure 3).

In nature, *Araneus diadematus* spiders store ADF-3 and ADF-4 proteins (MWs > 200 kDa) as highly concentrated (up to 50 wt%) solutions in a sac, known as the lumen, without the onset of undesirable aggregation[21] inside the spider. When necessary, the spider exposes the proteins to certain chemical and mechanical stimuli that trigger protein assembly into fibres in which the two proteins comprising the core filament are inhomogeneously distributed (see figure 2) (the origin of this inhomogeneous distribution is due to the difference in primary amino acid sequence of the two proteins).[19,20] The more 'crystalline' protein is distributed throughout the core of the lifeline fiber

forming β-sheet rich crystals, with the more 'elastic' protein in the core of the fiber forming a matrix. The core is coated in a layer of silk proteins produced in the minor ampullate gland, a glycoprotein coat, and finally a lipid coat (see figure 2).

Flagelliform silk

Silk fibers made of proteins produced in the flagelliform silk gland (Flag silk, MW ca. 500 kDa) are highly elastic and are used to produce the capture spiral. In comparison to MA silk, Flag silk is composed of only one major protein that contains greater amounts of proline and valine, and reduced amounts of alanine. Flag silk protein from *N. clavipes* is comprosed of blocks of $(GGX)_n$ that form 3_{10}-helices, blocks of GPGXX that form β-turn spirals (imparting elasticity/flexibility to the fibers), and a highly conserved non silk-like spacer sequence the function of which is uncertain, however, its polar hydrophilic nature suggest that it may be important for both cross-linking and hydration of the fiber.[8]

BIOTECHNOLOGICAL PRODUCTION OF SPIDER SILK PROTEINS

Inspired by the fantastic properties of spider silk proteins[22-25] we began research into their production in 2002.[22,26,27] Initially, we concentrated on developing a method for recombinantly producing proteins with primary structures identical to those of naturally occurring *Araneus diadematus* major ampullate silk proteins (ADF-3 and ADF-4) and *Nephila clavipes* flagelliform silk proteins. We used baculoviruses to transfer partial cDNAs coding for ADF-3, ADF-4 and flagelliform silk proteins (containing genetic information corresponding to the repetitive backbone and non-repetitive Ccarboxy-terminus of the proteins) into an insect cell line (Sf9 cells derived from the fall armyworm *Spodoptera frugiperda*) which we used as expression hosts for the silk proteins. Using this methodology we can successfully produce proteins (MWs of up to ca. 120 kDa) with accurate

primary structures, which has enabled us to carry out initial studies of the self-assembly properties of the proteins. However, we were deterred from scaling up the fermentations by the relatively low yield of ca. 30 mg per liter.[28,29]

Motivated by a desire to produce spider silk proteins at low cost on an industrial scale we decided to utilize *Escherichia coli* (*E. coli*) BLR(DE3) bacteria as host cells for the production of recombinant silk-like proteins, and have successfully designed and produced a variety of silk-like proteins based upon the major ampullate silks of *Araneus diadematus* spiders (ADF-3 and ADF-4) and *Nephila clavipes* flagelliform silk proteins. Our proteins (with molecular weights that are precisely controllable between 40 and 120 kDa) are obtained in high yield (> 500 mg per liter) by high density fermentation in *E. coli* and can be purified without the need for chromatographic separation which can be expensive and time consuming.[30] Whilst optimizing the fermentation process, we observed that although the BLR strain produces silk proteins during fermentations carried out in full media, it does not do so in cheaper minimal media due to an isoleucine auxotrophy (unless the media is supplemented with the respective amino acid); by use of an alternative strain of *E. coli* (HMS174(DE3) K-12 derivatives) we are now able to produce silk proteins by fermentation using minimal media which we regard as a significant step towards the industrial production of our proteins.[31]

SILK PROTEIN PROCESSING

Silk protein solubility

Naturally occurring spider silk fibers are highly insoluble in water (partially due to their relatively high β-sheet content physically cross-linking the proteins) and consequently require strongly denaturing conditions (such as 6M guanidinium thiocyanate which disrupts intra/inter-molecular

hydrogen bonds) to dissolve them.[19,20,28] Interestingly our recombinantly produced proteins with primary amino acid sequences identical to the natural proteins (produced by fermentation of Sf9 cells)[28] displayed remarkably different solubilities in water, with ADF-3 (the 'elastic' protein) being highly soluble (> 30 mg/mL) whereas ADF-4 (the 'crystalline' protein) was markedly less soluble (ca. 1 mg/mL). Our genetically engineered silk-like proteins (eADF-3 and eADF-4) produced by fermentation in *E. coli* have moderately improved solubilities as there are slightly fewer hydrophobic amino acids in the proteins.[30] Although water is a fantastic solvent for materials processing as it is readily available, cheap and biocompatible, for maximum versatility it is beneficial to be able to additionally use alternative solvents, and our silk-like proteins are luckily also highly soluble in non-aqueous solvents such as: formic acid; hexafluoroisopropanol and certain ionic liquids.[32] This versatility of solvent choice has facilitated their processing into various morphologies (including fibers, films, foams, hydrogels, spheres and capsules depicted in figure 4)[33], and the following sections of this highlight are devoted to their preparation.

Fibers

As outlined above, natural spider silk fibers have been used by mankind for applications as diverse as hunting (bow strings, cross-hairs, fishing lines or nets)[34] and wound healing (sutures/tissue scaffolds) due to their mechanical properties and biocompatibility. The preparation of artificial spider silk fibers with mechanical properties similar to natural spider silk fibers (from recombinantly produced spidroins) would allow the mass production of very tough fibers with clear application in textiles or wound healing.[27,35]

Spiders have evolved a highly efficient system utilising processes such as ion exchange (changing the relatively chaotropic sodium chloride for the strongly kosmotropic potassium phosphate), extraction of water, acidification and mechanical forces (elongational flow, shear and stretching),[36] for the

production of very tough β-sheet rich fibers (with μm scale diameters).[25] Inspired by this natural process, we added potassium phosphate to concentrated aqueous solutions (ca. 150 mg/mL) of our proteins and then applied mechanical shear (by pulling a fine metal rod from the viscous solution, a process known as hand-drawing) allowing us to produce smooth β-sheet rich fibers (with μm scale diameters – see figure 4).[37] More recently we have designed and manufactured a microfluidic device capable of producing β-sheet rich fibers by applying the principles outlined above,[38] and are currently investigating a variety of other methods of biomimetic fiber production (such as that depicted in figure 5) that will enable us to produce fibers (with μm and nm scale diameters) on an industrial scale.[39-41]

Hydrogels

Hydrogels are the subject of intense current research interest due to their potential application within cosmetics, drug delivery devices, food additives (rheology modification) and tissue scaffolds in tissue engineering. We have demonstrated that addition of potassium phosphate (< 300 mM) or methanol (ca. 10% v/v) to aqueous solutions of engineered spidroins (at concentrations of ca. 2 wt%) induces their self-assembly into β-sheet rich nanofibrils[42] that hierarchically assemble into a sample spanning network that ultimately immobilizes the solvating water, yielding a hydrogel. Such self-assembled hydrogels can be disrupted by agitation or shearing; consequently, chemical cross-linking of the hydrogels with ammonium peroxodisulfate and tris(2,2'-bipyridyl)dichlororuthenium was employed to produce robust and highly elastic hydrogels (see figure 4).[42,43] We are currently in the process of investigating spider silk-like protein based hydrogels for a number of different biomedical applications.

Spheres

The encapsulation and delivery of active ingredients (such as drugs, dyes, flavors or perfumes) is commonly achieved by formulation within polymer spheres. With this in mind we prepared solid protein spheres (with diameters tunable between nanometer and micrometer scale - see figure 4) by the addition of high concentrations of potassium phosphate (> 400 mM)[42,44,45] to solutions of our engineered spidroins. The β-sheet rich spheres form due to the liquid–liquid phase separation of a protein-rich phase in a protein-poor supernatant, owing to the potassium phosphate induced salting out of the protein-rich phase (inspired by the natural fiber spinning process). Sphere size can be controlled with two simple parameters, protein concentration and mechanical shear (in this case the mixing intensity of the protein solution with the potassium phosphate solution); increasing the protein concentration resulted in larger spheres, whereas increasing the mixing intensity resulted in smaller spheres.[45] Moreover, we have encapsulated hydrophobic compounds (e.g. β-carotene) within such β-sheet rich spheres, and clearly demonstrated that the spheres were undigested in artificial gastric fluid and completely digested in artificial intestinal fluid at 37 ^{o}C. This sort of controlled release highlights the potential of the protein spheres for use as drug delivery vehicles that remain intact in the stomach and release the encapsulated drug in the small intestine.[46]

Capsules

The encapsulation and delivery of active ingredients (such as drugs, dyes, flavors or perfumes) may also be achieved by formulation within polymer capsules. The inherently surfactant-like nature of our spider silk-like proteins causes them to spontaneously assemble at interfaces, such as those found between air/water or organic solvents/water.[47-50] We have utilized this property to prepare capsules (with tunable diameters) using water-in-oil (toluene) emulsions as templates, yielding β-sheet-rich capsules that are thin yet mechanically and chemically stable (see figure 4). The

microcapsules can be transferred into aqueous solution either via centrifugation into an aqueous sub-layer, or dilution of the toluene by the addition of an excess of ethanol and water.[51,52] The capsules are porous (with an average MW cutoff of ca. 27 kDa) therefore allowing small molecules (such as fluorescein – a simple model for a low molecular weight drug) to diffuse freely (resulting in a burst release), whereas macromolecules (such as FITC labeled dextran – a simple model for a macromolecular drug) are retained within the capsules (provided they are larger than the cutoff).[51] The capsules can be degraded upon exposure to Proteinase K in a matter of minutes. Interestingly this degradation can be prevented by chemically cross-linking the proteinaceous membrane of the capsules via photo-initiated oxidation with ammonium peroxodisulfate and tris(2,20-bipyridyl)dichlororuthenium(II).[52]

Films

Coatings are commonly applied to the surface of materials to modify their surface properties. The biocompatibility of our spider silk-like proteins should allow their application as coatings for biomedical implants. As noted above, our spider silk-like proteins are soluble in non-aqueous solvents such as hexafluoroisopropanol in which they tend to adopt an α-helical conformation, consequently films (such as those shown in figure 4) cast from solutions of recombinant spidroins in hexafluoroisopropanol tend to be α-helix rich and water soluble.[33] The as-cast films are smooth, and exposure to potassium phosphate or methanol induces β-sheet formation (rendering the films insoluble in water) and increases the surface roughness.[53,54] We have prepared multilayer films[32] and chemically modified the surface of such films via carbodiimide-mediated coupling of active enzymes (e.g. β-galactosidase) through the carboxylic acid groups displayed on the backbone of the spidroins. The retention of enzymatic activity of films displaying β-galactosidase on their surface was shown using 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside as a substrate.[55]

CONCLUSIONS

We have established a simple and effective method of producing and purifying spider silk-like proteins. Our spider silk-like proteins are soluble in both aqueous and non-aqueous solvents, which allows the preparation of a variety of materials morphologies (e.g. fibers, films, foams, hydrogels, spheres and capsules). We believe that in the future materials derived from our recombinantly produced spider silk-like proteins will be utilized for applications requiring large quantities of our proteins (e.g. textiles) and in high-tech biomedical applications (such as tissue scaffolds and drug delivery devices); and furthermore, that *de novo* designed spider silk-like proteins currently under development in our laboratories will be of great interest due to their highly tunable structures.

ACKNOWLEDGEMENTS

JGH gratefully acknowledges financial support from the Alexander von Humboldt Foundation, and TS acknowledges financial support from Army Research Office (W911NF-0810284).

REFERENCES AND NOTES

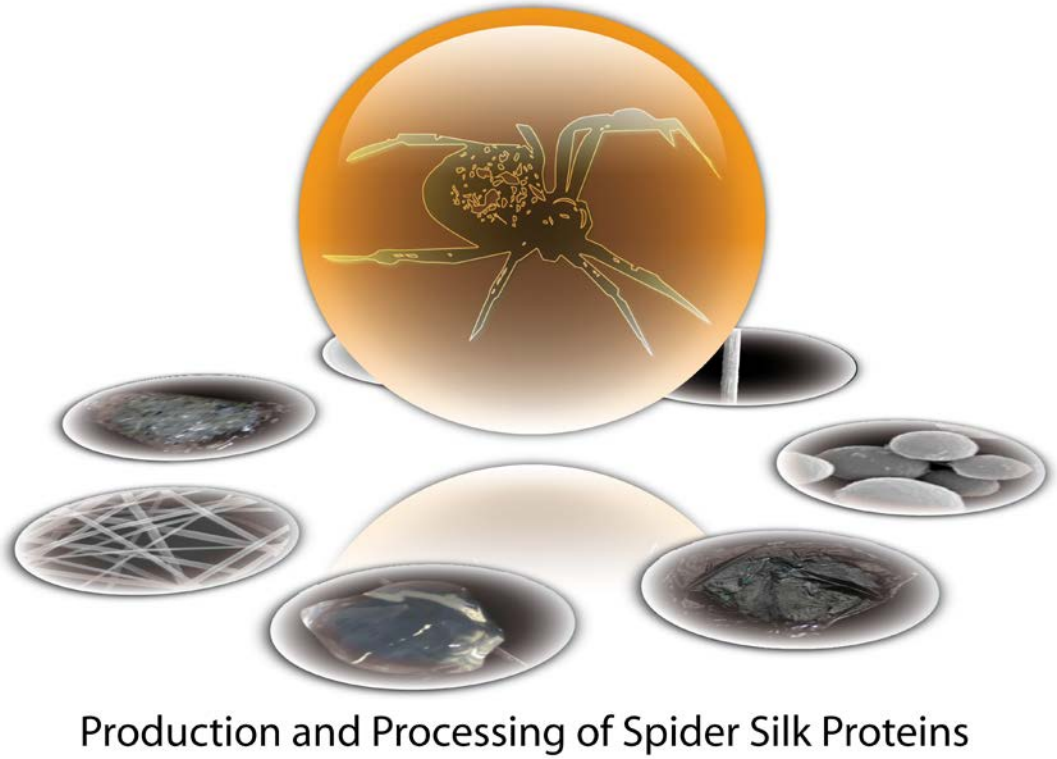
1. Gosline, J. M.; Demont, M. E.; Denny, M. W. Endeavour 1986, 10, 37-43.
2. Gosline, J. M.; Guerette, P. A.; Ortlepp, C. S.; Savage, K. N. Journal of Experimental Biology 1999, 202, 3295-3303.
3. Vollrath, F. Experientia 1985, 41, 1229-1229.
4. Aprhisiart, A.; Vollrath, F. Behavioral Ecology 1994, 5, 280-287.
5. Lin, L. H.; Edmonds, D. T.; Vollrath, F. Nature 1995, 373, 146-148.
6. Lewis, R. V. Chemical Reviews 2006, 106, 3762-3774.
7. Altman, G. H.; Diaz, F.; Jakuba, C.; Calabro, T.; Horan, R. L.; Chen, J. S.; Lu, H.; Richmond, J.; Kaplan, D. L. Biomaterials 2003, 24, 401-416.
8. Hayashi, C. Y.; Lewis, R. V. Journal of Molecular Biology 1998, 275, 773-784.
9. Hayashi, C. Y.; Lewis, R. V. Science 2000, 287, 1477-1479.
10. Hayashi, C. Y. American Zoologist 2001, 41, 1468-1468.
11. Hayashi, C. Y.; Lewis, R. V. Bioessays 2001, 23, 750-756.

12. Gatesy, J.; Hayashi, C.; Motriuk, D.; Woods, J.; Lewis, R. Science 2001, 291, 2603-2605.
13. Swanson, B. O.; Blackledge, T. A.; Summers, A. P.; Hayashi, C. Y. Evolution 2006, 60, 2539-2551.
14. Garb, J. E.; DiMauro, T.; Vo, V.; Hayashi, C. Y. Science 2006, 312, 1762-1762.
15. Ayoub, N. A.; Garb, J. E.; Tinghitella, R. M.; Collin, M. A.; Hayashi, C. Y. Expert Review of Medical Devices 2007, 4, 430-430.
16. Challis, R. J.; Goodacre, S. L.; Hewitt, G. M. Insect Molecular Biology 2006, 15, 45-56.
17. Augsten, K.; Weisshart, K.; Sponner, A.; Unger, E. Scanning 1999, 21, 77-77.
18. Sponner, A.; Schlott, B.; Vollrath, F.; Unger, E.; Grosse, F.; Weisshart, K. Biochemistry 2005, 44, 4727-4736.
19. Sponner, A.; Unger, E.; Grosse, F.; Klaus, W. Nature Materials 2005, 4, 772-775.
20. Sponner, A.; Vater, W.; Monajembashi, S.; Unger, R. E.; Grosse, F.; Wiesshart, K. PLoS ONE 2007, 2, e998.
21. Scheibel, T.; Buchner, J. Handbook of Experimental Pharmacology 2006, 172, 199-219.
22. Scheibel, T. Microbial Cell Factories 2004, 3, 14.
23. Scheibel, T. Current Opinion in Biotechnology 2005, 16, 427-433.
24. Romer, L.; Scheibel, T. Prion 2008, 2, 154-161.
25. Heim, M.; Keerl, D.; Scheibel, T. Angewandte Chemie-International Edition 2009.
26. Vendrely, C.; Scheibel, T. Macromolecular Bioscience 2007, 7, 401-409.
27. Romer, L.; Scheibel, T. Chemie in Unserer Zeit 2007, 41, 306-314.
28. Huemmerich, D.; Scheibel, T.; Vollrath, F.; Cohen, S.; Gat, U.; Ittah, S. Current Biology 2004, 14, 2070-2074.
29. Vendrely, C.; Ackerschott, C.; Romer, L. M.; Scheibel, T. In Nanostructure Design: Methods and Protocols; Gazit, E.; Nussinov, R., Eds.; Humana Press: Totowa, 2008, p 3-14.
30. Huemmerich, D.; Helsen, C. W.; Quedzuweit, S.; Oschmann, J.; Rudolph, R.; Scheibel, T. Biochemistry 2004, 43, 13604-13612.
31. Schmidt, M.; Romer, L.; Strehle, M.; Scheibel, T. Biotechnology Letters 2007, 29, 1741-1744.
32. Junghans, F.; Morawietz, M.; Conrad, U.; Scheibel, T.; Heilmann, A.; Spohn, U. Applied Physics a-Materials Science & Processing 2006, 82, 253-260.
33. Hardy, J. G.; Romer, L. M.; Scheibel, T. R. Polymer 2008, 49, 4309-4327.
34. Gerritsen, V. B. Protein Spotlight 2002, July.
35. Lammel, A.; Keerl, D.; Romer, L.; Scheibel, T. In Recent Advances in Biomaterials Research; Hu, J., Ed.; Transworld Research Network: Kerala, 2008, p 1-22.
36. SenGupta, S.; Scheibel, T. In Protein Folding-Misfolding: Some Current Concepts of Protein Chemistry; Zbilut, J. P.; Scheibel, T., Eds.; Nova Publishers: New York, 2007, p 1-34.
37. Exler, J. H.; Hummerich, D.; Scheibel, T. Angewandte Chemie-International Edition 2007, 46, 3559-3562.
38. Rammensee, S.; Slotta, U.; Scheibel, T.; Bausch, A. R. Proceedings of the National Academy of Sciences of the United States of America 2008, 105, 6590-6595.
39. Hardy, J. G.; Romer, L.; Scheibel, T. Polymer 2008, 49, 4309-4327.
40. Romer, L.; Scheibel, T. Nachrichten Aus Der Chemie 2008, 56, 516-519.
41. Scheibel, T. R.; Serpell, L. In Protein Folding Handbook. Part II.; Buchner, J.; Kiefhaber, T., Eds.; Wiley-VCH: Weinheim, 2004, p 193-249.
42. Slotta, U.; Hess, S.; Spiess, K.; Stromer, T.; Serpell, L.; Scheibel, T. Macromolecular Bioscience 2007, 7, 183-188.
43. Rammensee, S.; Huemmerich, D.; Hermanson, K. D.; Scheibel, T.; Bausch, A. R. Applied Physics a-Materials Science & Processing 2006, 82, 261-264.
44. Slotta, U. K.; Rammensee, S.; Gorb, S.; Scheibel, T. Angewandte Chemie-International Edition 2008, 47, 4592-4594.
45. Lammel, A.; Schwab, M.; Slotta, U.; Winter, G.; Scheibel, T. ChemSusChem 2008, 1, 413-416.

46. Liebmann, B.; Huemmerich, D.; Scheibel, T.; Fehr, M. Colloids and Surfaces a-Physicochemical and Engineering Aspects 2008, 331, 126-132.
47. Geisler, M.; Pirzer, T.; Ackerschott, C.; Lud, S.; Garrido, J.; Scheibel, T.; Hugel, T. Langmuir 2008, 24, 1350-1355.
48. Horinek, D.; Serr, A.; Geisler, M.; Pirzer, T.; Slotta, U.; Lud, S. Q.; Garrido, J. A.; Scheibel, T.; Hugel, T.; Netz, R. R. Proceedings of the National Academy of Sciences of the United States of America 2008, 105, 2842-2847.
49. Zbilut, J. P.; Scheibel, T.; Huemmerich, D.; Webber, C. L.; Colafranceschi, M.; Giuliani, A. Applied Physics a-Materials Science & Processing 2006, 82, 243-251.
50. Zbilut, J. P.; Scheibel, T.; Huemmerich, D.; Webber, C. L.; Colafranceschi, M.; Giuliani, A. Physics Letters A 2005, 346, 33-41.
51. Hermanson, K. D.; Harasim, M. B.; Scheibel, T.; Bausch, A. R. Physical Chemistry Chemical Physics 2007, 9, 6442-6446.
52. Hermanson, K. D.; Huemmerich, D.; Scheibel, T.; Bausch, A. R. Advanced Materials 2007, 19, 1810-1815.
53. Slotta, U.; Tammer, M.; Kremer, F.; Koelsch, P.; Scheibel, T. Supramolecular Chemistry 2006, 18, 465-471.
54. Metwalli, E.; Slotta, U.; Darko, C.; Roth, S. V.; Scheibel, T.; Papadakis, C. M. Applied Physics a-Materials Science & Processing 2007, 89, 655-661.
55. Huemmerich, D.; Slotta, U.; Scheibel, T. Applied Physics a-Materials Science & Processing 2006, 82, 219-222.

LEGENDS FOR PROFILES AND FIGURES

Cover illustration. The production and processing of spider silk proteins: we produce Spider silk-like proteins using biotechnology, and subsequently utilise biomimetic processes to prepare useful materials morphologies that have a variety of technical and biomedical applications.



Profile 1. John Hardy

Profile 2. Thomas Scheibel

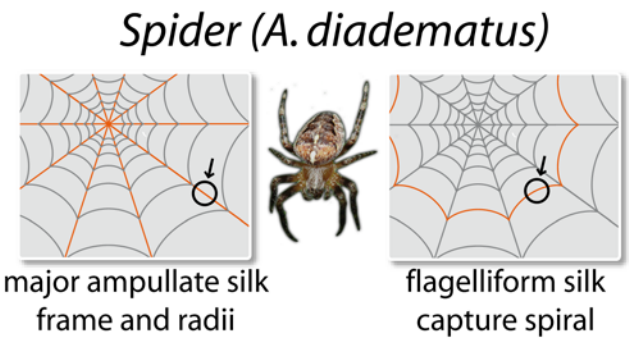


Figure 1. The two most important silks (major ampullate and flagelliform silk fibers) in orb webs spun by *Araneus diadematus* spiders.

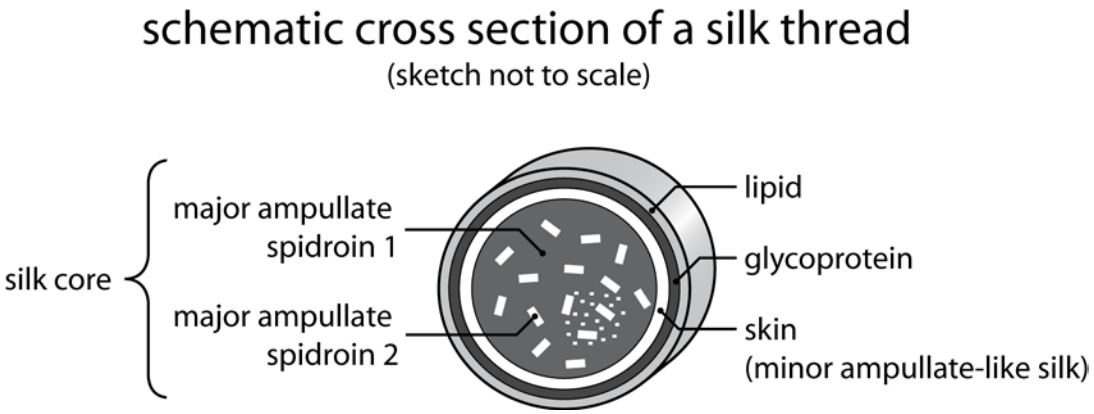


Figure 2. Schematic representation of a cross section of major ampullate silk fibers.

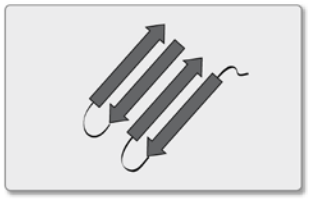
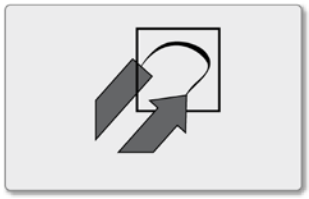
Structure	Illustration	Amino acid sequence
random coil		various
α-helix		NR_N / NR_C regions and A_n (in solution)
3_{10}-helix		(GGX)
ß-sheet		$(GA)_n$ / A_n
ß-turn		(GPGQQ) / (GPGGX)

Figure 3. The repetitive blocks of amino acids that give rise to the common secondary structural motifs in spider silk proteins.

Figure 4. The various morphologies that we have prepared with our bioengineered silk proteins.

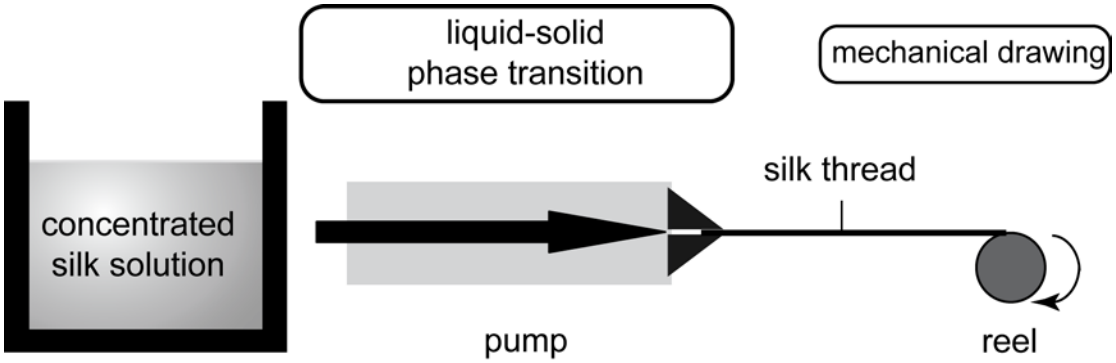
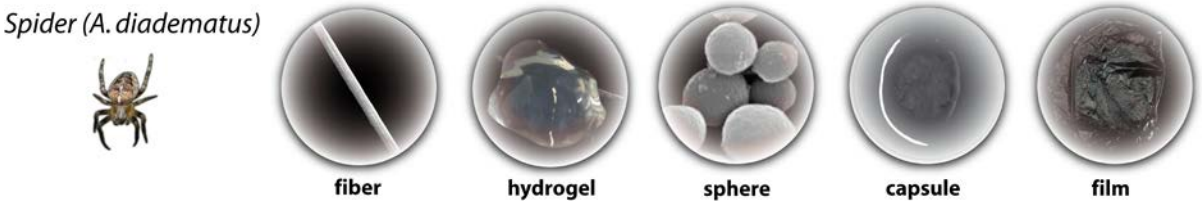


Figure 5. Biomimetic silk fiber spinning process.



Graphical abstract